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ION AND WATER BALANCE OF THE HYPO- AND HYPEROSMOTICALLY STRESSED CHITON *MOPALIA MUSCOSA*

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Molluscs inhabiting rocky intertidal zones and estuaries often experience salinity stress, as freshwater run-off after heavy rain (Boyle, 1969) or tidal fluctuations of salinity (Stickle and Ahokas, 1975). A rapid reduction of external salinity causes these organisms to gain water osmotically, with the result that physiological functions such as respiration, feeding, and locomotion may be severely impaired (Oglesby, 1975). The water taken up can be considered to enter both the extracellular space (in the case of animals with open circulatory systems, the blood) and the intracellular space. Volume regulation of both is required for survival.

Cell volume regulation has been extensively investigated in marine and estuarine invertebrates (Pierce and Greenberg, 1973). Control of cell volume occurs through changes in the concentration of intracellular free amino acids (FAA). For example, during hyposmotic stress these acids leave the cell intact followed by osmotically obligated water, and cell volume is restored (reviewed by Watts and Pierce, 1978).

Much less is known about whole-animal volume regulation of molluscs. This aspect of volume regulation is complex. It may simultaneously involve salt and water movements between the animal's body fluids and the medium, shifts of free amino acids between cells and extracellular fluids (cell volume regulation), change of osmotic permeability, and change in excretion rates (Fletcher, 1974b). In addition, these mechanisms may contribute to volume control at different times after exposure of the organism to hyposmotic sea water.

Most work on volume regulation of molluscs has been performed with bivalves and gastropods. Both groups have an encompassing shell which makes accurate wet-weight determinations difficult. The polyplacophoran molluscs, the chitons, do not have an encompassing shell. Instead, they have eight serially arranged plates embedded in the girdle on their dorsal surface. In addition, chitons readily attach themselves to petri dishes, facilitating weighing of the animal. The chiton's wet weight is determined by subtracting the weight of the petri dish.

The chiton *Mopalia muscosa* inhabits the rocky intertidal zone of the Pacific coasts of the United States and Canada. The purpose of this study was to characterize whole-animal volume regulation in *M. muscosa*. We also report the blood-ion concentrations and muscle-tissue water content of chitons acclimated to different salinities.

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Materials and Methods

Collection of animals and maintenance conditions

Specimens of *Mopalia muscosa* were collected on the south jetty of Elkhorn Slough, Moss Landing, California. All chitons were taken within the intertidal zone, and between −0.3 and +0.6 m of mean lower low water. Collections were restricted to this intertidal range to limit intraspecific differences resulting from different habitats. At the laboratory, the chitons were placed in a maintenance tank which held aerated normal sea water (33.3–34.5‰ salinity; pH =7.7–8.1) at 13°C ±2°. All chitons were allowed 6–13 days to adjust to these conditions before experiments were performed. The chitons ranged in weight from 5 to 22 g.

Source and preparation of experimental SW

Sea water was obtained from the mouth of Elkhorn Slough at high tide, filtered with a Glass Fiber Type A filter, and approximately 11 g of an artificial sea-salt mixture (Instant Ocean) was added to a liter of filtered sea water (SW) in order to raise the salinity to 42.1‰ (125% SW); lower salinities were made by diluting the 125% SW with deionized water. The salinity of all solutions was determined before use with an induction salinometer (± 0.01‰).

Acclimation of chitons to experimental SW

Most experiments were conducted at 13.3°C; the pH varied between 8.0 and 8.3. The effect of salinity on blood-ion concentrations was examined by acclimating batches of three chitons to experimental media of 125, 100, 75, and 60% SW for 48 hr. Then a sample of hemolymph was removed from each damp-dried chiton by puncturing the body wall medially just beneath the eighth valve with a needle and 1 cc syringe. The pericardium is near the region where hemolymph was obtained. However, pericardial fluid would be expected to be colorless when withdrawn, due to ultra-filtration of hemolymph through the heart wall. In each case blue fluid was obtained, indicating the presence of hemocyanin, and assuring that the fluid was blood and not pericardial fluid. The hemolymph collected from each animal was centrifuged to remove cells. The supernatants were used for ion-concentration determinations.

The effect of hyposmotic SW on the kinetics of change of chiton blood Na^+ and Cl^- concentrations was investigated by placing chitons in 60% SW and then removing three chitons at 2, 4, 6, 8, 12, and 24 hr for hemolymph-ion analysis. Three chitons from the maintenance tank served as time 0 samples.

Measurement of SW and blood-ion concentrations

Sodium, K^+, Ca^{2+}, and Mg^{2+} concentrations of hemolymph and SW were determined by atomic-absorption spectrophotometry (Perkin-Elmer 305B). Sodium, Ca^{2+}, and Mg^{2+} concentrations were measured by diluting hemolymph and SW appropriately with 1000 ppm K^+, in order to prevent ionization effects, in 1% HNO_3; the diluting solution for the Ca^{2+} and Mg^{2+} determinations also contained 1% La^{3+} to prevent phosphate interference with Ca^{2+} and Mg^{2+} determinations. The diluting solution for determination of K^+ concentrations contained 1000 ppm Na^+, again to prevent ionization effects, in 1% HNO_3. Standards were prepared with the appropriate diluting solution. Chloride concentrations were determined

with an Oxford Titrator by the mercuric titration method of Schales and Schales (1941).

Measurement of wet-weight changes as an indicator of volume regulation

Chitons were placed in the maintenance SW on preweighed plastic petri dishes and allowed 2–3 days to attach to them. The petri dish plus the chiton was weighed and then placed into 2 l of aerated experimental SW and weighed at 1, 2, 4, 6, 8, 12, and 24 hr. Wet weights were determined by removing the petri dish with the attached chiton from the SW, blotting dry, and immediately weighing on an analytical balance to the nearest 0.01 g. Nine replicate weighings of a single chiton in 100% SW gave a standard deviation of ±0.8% body wet weight. The small volume of water trapped in the mantle cavity of the chitons was assumed to remain unchanged during the experiment. The resulting small overestimates of the chiton's true wet weight were too small to seriously affect conclusions about volume regulation.

Lange and Mostad (1967) have criticized volume-regulation experiments using whole animals: They state that weight variations could be caused by excretion and loss of fecal pellets. Few fecal pellets were seen in the containers during volume-regulation experiments with *M. muscosa;* defecation probably did not contribute to the variability in wet-weight determinations. However, excretion remains a possible source of variation.

The effect of temperature on volume regulation was studied by holding the chitons for 2–3 hr in 100% SW at either 7° or 19°C and then placing them in 60% SW of the same temperature and monitoring wet weights at 0, 2, 4, 6, 8, 12, and 24 hr.

Calculations of osmotic permeability (P_{os}) and of chitons as osmometers

Calculations of osmotic permeability, P_{os}, and the theoretical rate of weight change of chitons responding as if they were perfect osmometers were based on the wet weight of the soft parts, determined as follows. Sixteen chitons were blotted dry and their wet weight determined by weighing on an analytical balance. Next, the chitons' plates were removed by scraping all girdle tissue from the plates, and all eight plates were weighed. Then, a regression line of plate wet weight *vs.* total-body wet weight was calculated. The equation was $Y = 0.34 - 0.6X$, and r, the coefficient of correlation, was 0.998. Thus plate wet weight could be obtained from a measurement of total-body weight. The wet weight of the soft parts could then be found by subtracting the plate wet weight from the total-body wet weight.

In order to base all P_{os} values and theoretical values for chitons responding as perfect osmometers on the soft-part water content, the total body water content had to be determined. This was done by drying four chitons to constant weight at 67°C. Using the above regression equation, the soft-part water content of the chitons could be determined.

The osmotic water permeability, P_{os}, was calculated according to Fletcher (1974a):

$$P_{os} = \frac{-1}{t(C_1 + C_o' - C_o)}\left[f + \frac{Z_o C_o'}{C_1 + C_o' - C_o} \times \log_e\left\{1 - \frac{f(C_1 + C_o' - C_o)}{Z_o(C_o - C_1)}\right\}\right] \quad \text{(Equation 1)}$$

where P_{os} is in kg H_2O/kg animal × hr × unit osmolal concentration difference, C_o is the osmolality of the acclimatization medium (100% SW), C_o' the osmolality of chiton hemolymph in the acclimating medium, Z_o the water content of the animal (based on soft parts) (kg H_2O/kg animal when acclimated to C_o), C_1 is the osmolality of the experimental medium (60% SW) and f is the fractional increase in weight t hrs after transfer to the experimental medium. Whenever the term $C_o' - C_o$ occurred in the equation it was cancelled, since chitons apparently are osmoconformers (McGill, 1975; Simonsen, 1975; Boyle, 1969).

At various times after the chitons were placed in 60% SW, f was measured. P_{os} was calculated for each time, and a graph of P_{os} against t plotted.

The theoretical increase in the weight of soft parts at a given time t of chitons behaving as if they were perfect osmometers was calculated according to Fletcher (1974b):

$$t = \frac{1}{P_{os}(C_1 + C_o' - C_o)} \left\{ \frac{Z_o C_o'}{C_1 + C_o' - C_o} \times \log_e \left[\frac{Z_o(C_o - C_1)}{Z_o(C_o - C_1) - f(C_1 + C_o' - C_o)} \right] - f \right\} \quad \text{(Equation 2).}$$

Initial values of P_{os} must be used to calculate the theoretical osmometer curves; later values would be reduced by the mechanisms regulating chiton volume. The initial values were obtained from the plot of P_{os} against time.

Determination of muscle-tissue water content

After 9–10 chitons had acclimated for 48 hr to the various experimental media, the muscle-tissue water content was determined. Small portions of foot muscle (60–110 mg) were oven dried at 96°C for 20 hr, and the percent water content was obtained from the difference between the wet and dry weights. These data were arcsine transformed for statistical purposes (Sokal and Rohlf, 1969). However, the untransformed data are presented graphically.

Statistical methods

Means of two treatment groups were compared by Student's *t* test after determining homogeneity of variance with an *F* test (Sokal and Rohlf, 1969). In a few cases the non-parametric Mann Whitney *U* test was used to test for statistical differences between two treatment groups. Regression analysis was performed by the method of least squares, and the significance of the regression coefficient was established by a *t* test (Sokal and Rohlf, 1969). Statistical significance is considered to be the 5% level of probability.

Results

Effect of salinity on hemolymph ion concentrations

Following acclimation, the hemolymph Na^+ and Cl^- concentrations of *Mopalia muscosa* were equivalent to the SW concentrations at all salinities tested. Hemolymph K^+ and Mg^{2+} concentrations were also isoionic to the SW, except at 60% SW where the hemolymph K^+ and Mg^{2+} concentrations were greater than the SW values ($P < 0.05$, K^+; $P < 0.05$, Mg^{2+}, Mann Whitney *U* test). The blood Ca^{2+}

TABLE I

Blood ion concentrations of M. muscosa *(mEq/l), at different salinities, compared to the SW concentration. N = 3 for each ion at each salinity. Values are expressed as the mean ±SE.*

	% SW	Blood	Sea Water
Cl^-	125	673.2 ± 10.00	686.2 ± 3.70
	100	531.2 ± 9.10	543.5 ± 0.60
	75	407.5 ± 1.70	402.4 ± 2.10
	60	343.7 ± 3.80	348.1 ± 1.10
Na^+	125	590.8 ± 3.50	604.5 ± 1.00
	100	483.2 ± 13.00	479.2 ± 6.00
	75	374.9 ± 5.10	377.0 ± 8.10
	60	303.4 ± 4.90	318.8 ± 6.00
K^+	125	11.6 ± 0.60	11.0 ± 0.25
	100	10.2 ± 0.50	8.6 ± 0.20
	75	7.3 ± 0.12	6.9 ± 0.05
	60	5.8 ± 0.07*	5.5 ± 0.05
Ca^{2+}	125	27.3 ± 0.50	26.7 ± 0.10
	100	21.8 ± 0.29	20.8 ± 0.01
	75	17.5 ± 0.13**	14.8 ± 0.00
	60	15.4 ± 0.18**	12.7 ± 0.01
Mg^{2+}	125	119.5 ± 2.75	122.9 ± 0.20
	100	96.9 ± 1.18	97.2 ± 1.60
	75	75.2 ± 0.13	75.1 ± 0.20
	60	61.2 ± 0.21***	59.9 ± 0.00

* $P < 0.05$, hemolymph *vs.* SW (*t* test)
** $P < 0.001$, hemolymph *vs.* SW (*t* test)
*** $P < 0.05$, hemolymph *vs.* SW (Mann Whitney *U* test)

concentration of chitons acclimated to 125% and 100% SW were equal to that of SW; but the hemolymph Ca^{2+} concentration was hyperionic in chitons acclimated to 75 and 60% SW (Table I).

Hemolymph Cl^- concentration of chitons transferred to 60% SW from 100% SW reached equilibrium with the 60% SW Cl^- concentration 8 hrs after transfer. Blood Na^+ concentration came into equilibrium between 8 and 12 hr after transfer of chitons from 100 to 60% SW (Fig. 1).

Volume regulation

In the fall, *M. muscosa* regulates volume in salinities as low as 60% SW. Chitons exposed to 60% SW for 6 hr showed a maximum increase of approximately 20% in total-body wet weight, whereas chitons exposed to 75% SW for 2 hr showed a maximum increase of 10%. In both 60 and 75% SW there is a decline of 6–7% total-body wet weight after the maximum weight gain. In addition, the rates of weight loss were equivalent in both hyposmotic salinities (Fig. 2). A regression of maximum weight gain of fall chitons exposed to 60% SW on total-body wet weight showed no correlation between chiton size and maximum weight gain.

Chitons returned to 100% SW following exposure to 60 or 75% SW showed a 3–4% undershoot of the original wet weight during the first hour of exposure

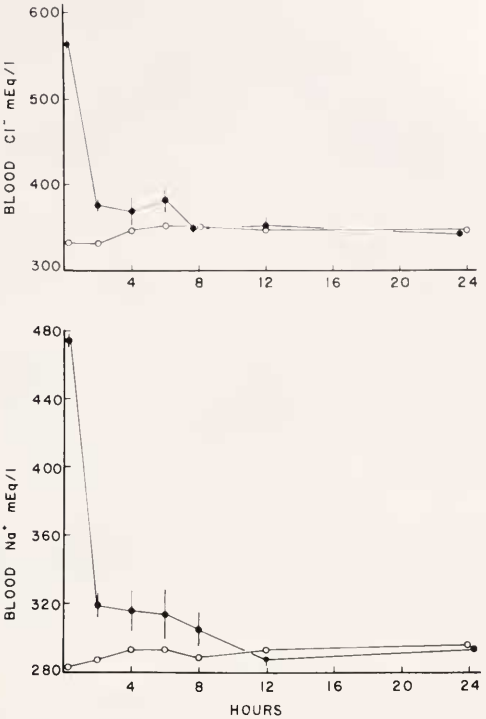

FIGURE 1. Solid circles indicate the effect of time and salinity on blood Na^+ and Cl^- concentration of *M. muscosa*. Chitons were transferred from 100 to 60% SW at time zero. Each point represents the mean, and vertical bars ± 1 SE, of three chitons. Open circles = 60% SW Na^+ and Cl^- concentration.

(Fig. 2). Two to four hr after transfer to 100% SW the undershoot peaked at approximately 6% loss of body weight. Over the next 20 hr a gradual but significant ($P < 0.01$, 60% SW; $P < 0.05$, 75% SW) increase in weight occurred. Chitons exposed to 125% SW lost 8–9% of their original total-body weight and showed limited volume regulation after 12 hr exposure to the hyperosmotic medium.

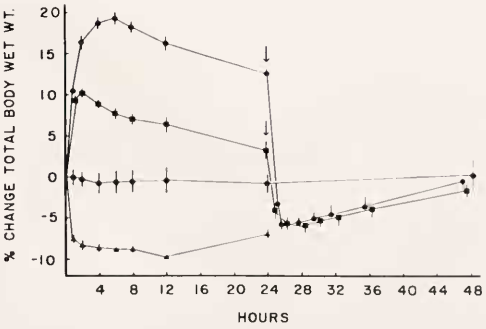

FIGURE 2. Volume regulation of *M. muscosa* in fall at different salinities. Chitons in 60% SW, N = 6 (solid circles); in 75% SW, N = 6 (solid squares); in 100% SW, N = 4 (solid hexagon); in 125% SW, N = 3 (solid triangle). Vertical bars indicate ± 1 SE. At the arrows the chitons exposed to 60 and 75% SW were returned to 100% SW.

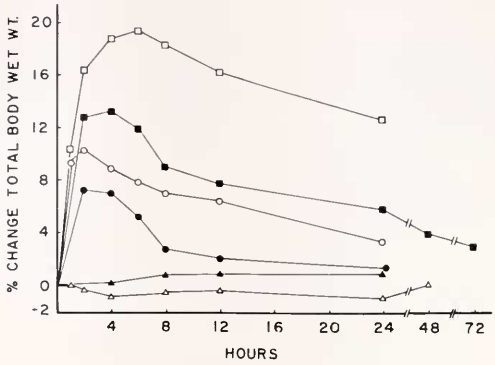

FIGURE 3. Volume regulation of *M. muscosa*. Spring chitons in 100% SW, N = 5 (solid triangles); in 75% SW, N = 7 (solid circles); in 60% SW, N = 5 (solid squares). Fall chitons in 100% SW (open triangles), in 75% SW (open circles) and in 60% SW (open squares). Sample size of fall chitons is shown in Fig. 2. Error bars are omitted for clarity.

Another set of volume regulation experiments were performed over 72 hr to determine if *M. muscosa* was capable of further reducing its weight if specimens spent more than 24 hr in 60% SW. These experiments were done in the spring. An additional weight reduction of only 2% occurred over the next 48 hr (Fig. 3). The volume regulation response of fall chitons is also shown in Figure 3 to illustrate the difference in volume regulation of fall and spring chitons. Fall chitons in both 60 and 75% SW gain significantly ($P < 0.01$, both salinities) more weight than spring animals. In addition, spring chitons regulate volume more quickly than do fall chitons in both 60 and 75% SW.

High temperature, 19°C, did not affect volume regulation of *M. muscosa* in 60% SW. However, low temperature, 7°C, reduced the chitons' ability to regulate volume (Fig. 4). Significant differences between the 7° and 13°C curves occurred at 2 ($P < 0.02$), 8 ($P < 0.05$), and 12 hr ($P < 0.05$). The 6 hr values were not significantly different because of the large amount of variation associated with the 6 hr value of the control curve, 13°C.

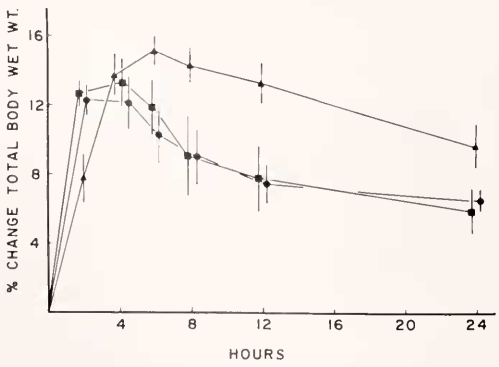

FIGURE 4. Volume regulation of *M. muscosa* in 60% SW at three temperatures in the spring. Chitons at 13°C, N = 5 (solid squares); 7°C, N = 6 (solid triangles); 19°C, N = 6 (solid circles). Data points are offset on the ordinate for clarity of diagram.

Osmotic permeability and chitons responding theoretically as osmometers

The osmotic permeability, P_{os}, (calculated according to equation 1) was 0.71 ± 0.045 kg H_2O/kg animal × hr^{-1} × unit osmolal concentration difference after 1 hr of exposure of chitons to 60% SW; the 2 hr value was 0.66 ± 0.05. These P_{os} values did not differ significantly and were averaged and used to calculate the theoretical rate of weight change (equation 2) of chitons responding as if they were perfect osmometers.

M. muscosa does not respond as an osmometer in hyposmotic SW. The percent increase of soft-part wet weight of chitons behaving as if they were simple Boyle-van't Hoff osmometers is 46.4%; this value agrees well with the asymptotic value of 48.7% calculated according to equation 2. The curve of chitons responding as if they were osmometers and the curve of observed weight change begin to separate after approximately 1 hr. Therefore, mechanisms of volume control are coming into effect within 1 hr after transfer of chitons to 60% SW (Fig. 5).

Muscle tissue water content

With acclimation (2 days) to decreasing salinity the foot-muscle tissue of *M. muscosa* becomes increasingly hydrated. However, this tissue does not appear to respond as a Boyle-van't Hoff osmometer ($P < 0.001$, 60% SW; $P < 0.02$, 75% SW; $P < 0.01$, 125% SW) (Fig. 6).

Discussion

The response of blood ions to changes in external salinity shows that the chiton *Mopalia muscosa* does not regulate the Na^+ and Cl^- concentrations of its blood in either hyposmotic (60% SW) or hyperosmotic (125% SW) media. Although the K^+ and Mg^{2+} concentrations of the blood of 60% SW acclimated chitons are greater than the respective SW concentrations, these differences are small.

The blood Ca^{2+} concentration of hyposmotically stressed chitons is hyperregulated. Regulation of blood Ca^{2+} concentration has been shown in other hyposmotically stressed molluscs, such as the limpet *Acmaea* (= *Notoacmea*)

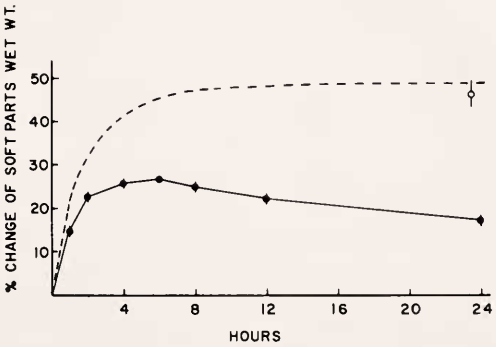

Figure 5. Comparison of the rate of weight change of fall *M. muscosa* responding, theoretically, as osmometers (dashed line) with the observed rate of weight change (solid circles), N = 6, vertical bars = ± 1 SE. All data are based on wet weight of the soft parts. Predicted steady-state weight change of chitons responding as if they were osmometers (open circle).

scutum from estuarine environments (Webber and Dehnel, 1968) and the chiton *Nuttalina californica* (Piper, 1975). The significance of hyper-regulation of blood Ca^{2+} concentration in molluscs is not known, but it could be of possible physiological benefit to the organism. Extracellular Ca^{2+} is involved in release of neurotransmitters from presynaptic neurons of the squid *Loligo vulgaris* (Miledi, 1973), and the action potential in the heart of the bivalve *Modiolus demissus* is dependent primarily on Ca^{2+} (Wilkens, 1972). Furthermore, ciliary movement is dependent on extracellular Ca^{2+} concentration (Eckert, 1972; Murakami and Eckert, 1972).

The blood Na^+ and Cl^- concentrations are reduced most rapidly during the first 2 hr of exposure to 60% SW (Fig. 1). The reduction of blood salt concentration could be due to salt loss (either diffusive across the body wall or through the urine) and to dilution of the blood by the water influx. The relative importance of salt loss and water influx in reducing blood Na^+ and Cl^- concentrations remains uncertain but cannot be ignored, since the diffusive salt loss would contribute to volume control by reducing the driving force for osmotic water flux existing across the body wall of hyposmotically stressed chitons. Diffusive salt loss in chitons has been demonstrated indirectly. Specimens of *Sypharochiton pelliserpentis* exposed to a seawater/sucrose mixture isosmotic to SW lost weight. This suggests that an isosmotic solution of salt and water was lost from the chitons, and that diffusive salt loss may contribute to volume control (Boyle, 1969).

Although blood osmotic pressure was not measured, the blood ion data, especially that of blood Na^+ and Cl^- concentrations, of salinity stressed chitons suggest that *M. muscosa* is an osmoconformer. Osmoconformity has been demonstrated in members of the genus *Mopalia* (Boyle, 1969); and the chitons *Cyanoplax hartwegii* (McGill, 1975), *Nuttalina californica* (Simonsen, 1975), and *Sypharochiton pelliserpentis* (Boyle, 1969) are osmoconformers in hypo- and hyperosmotic SW.

Since chitons are probably osmoconformers, intracellular volume regulation must occur during salinity stress. In most marine and estuarine invertebrates intracellular free amino acids are the source of solute for this regulation (Pierce

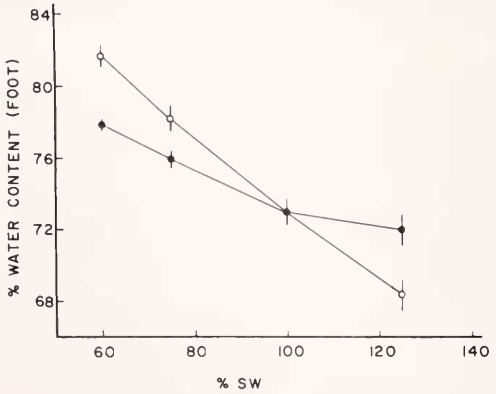

FIGURE 6. Water content of the foot muscle of *M. muscosa* as a function of salinity. Observed water content of muscle (closed circles); expected water content of muscle responding as if it were an osmometer (open circles). For chitons in 60, 75, and 100% SW, N = 10; 125% SW, N = 9. Vertical bars = ± 1 SE.

and Greenberg, 1973). Thus, osmotically stressed muscle tissue which relies on intracellular volume regulation would not be expected to alter its water content as would a Boyle-van't Hoff osmometer. This was found for the foot muscle-tissue of *M. muscosa* (Fig. 6).

Volume regulation has been studied in other chitons and in some bivalves. For example, the chiton *Cyanoplax hartwegii* can regulate volume in both 75 and 125% SW although the volume response is faster and more complete in 75% SW than in the hyperosmotic medium (McGill, 1975). McGill considers the hyposmotic volume-control response adaptive in chitons because it will limit swelling of the foot and allow continued attachment to the substrate. Volume regulation has also been studied in vertically separated populations of the chiton *Nuttalina californica*. Low-intertidal *Nuttalina* gain more weight in 50% SW than do high-intertidal specimens. Volume regulation was not demonstrated in either group; however, the maximum weight increase of both populations was less than that of chitons responding as if they were perfect osmometers (Simonsen, 1975). Likewise, the chiton *Sypharochiton pelliserpentis* does not regulate volume in hyposmotic SW. Instead, adaptation to hyposomotic media is accomplished by diffusive salt loss and by considerable tolerance to dilution of body fluids (Boyle, 1969). Volume regulation in hyposmotic SW has also been demonstrated in the bivalves *Modiolus demissus granosissimus* and *M. squamosus*. Volume control was not shown in either species when they were exposed to hyperosmotic SW (Pierce, 1971).

After a 24 hr exposure to either 60 or 75% SW (fall experiments), the chitons were returned to 100% SW, where they exhibited an undershoot of their original weight; that is, on return to 100% SW the chitons lost weight until they weighed less than their original weight at time zero. This may be due to loss of solute from the organism during exposure to hyposmotic SW (Gross, 1954). However, if this were the only cause the extent of undershoot should be correlated with salinity. This correlation was not found, suggesting that the chitons exposed to 60 and 75% SW lost the same amount of solute.

A somewhat surprising result of returning the chitons to 100% SW after exposure to hyposmotic SW was their steady weight gain after peak weight loss (Fig. 2). These chitons appear to begin volume regulation within 6 hr on return to 100% SW, whereas chitons exposed to 125% SW shrink passively and show limited ability to regulate volume. Thus, previous exposure to hyposmotic SW appears to activate volume regulation of *M. muscosa* re-exposed to 100% SW. These results differ from those of Pierce (1971) in a study of volume regulation in various species of *Modiolus*. Re-exposure of *Modiolus demissus granosissimum, M. modiolus, M. demissus,* and *M. squamosus* to full-strength SW after exposure to hyposmotic SW resulted in an undershoot of their original weight, and none of the species examined was able to regain its original volume. The physiological basis for this difference between chitons and bivalves is not known.

Volume control in an organism exposed to hyposmotic media may begin before the usually observed decline in weight. For example, *M. muscosa* begins to limit its rate of weight increase after 1 hr of exposure to hyposmotic SW (Fig. 5). Therefore, volume control mechanisms in *Mopalia* begin to function several hours before the observed decline in weight. Likewise, Machin (1975), using a graphic analysis of weight and water-content regulation, demonstrated that volume control in the polychaete *Glycera dibranchiata* begins before osmotic equilibrium is reached. The volume control mechanisms responsible for the chitons' early deviation from

perfect osmometer behavior are not known. However, this effect could result from a change in epithelial permeability to water, an increase in water excretion or diffusive salt loss.

Low temperature (7°C) reduced rate of weight gain of *M. muscosa* exposed to 60% SW. Likewise, the weight-loss rate was reduced during volume regulation. Therefore, temperature seems to affect processes controlling both osmotic influx and volume regulation.

Volume regulation in *Mopalia* may vary with season (Fig. 3). Chitons on the U. S.-Canadian West Coast reproduce in the spring, and this difference could be related to the animals' reproductive state. Furthermore, all chitons in this study were taken within a restricted vertical range in the intertidal zone: Animals separated vertically in the intertidal zone have different periods of exposure, and, as mentioned above, Simonsen (1975) has demonstrated that low-intertidal specimens of *Nuttalina californica* gain more weight in hyposmotic SW than high-intertidal animals. Thus, season and vertical position in the intertidal zone, should be considered in future studies concerning water balance of intertidal organisms.

In conclusion, *M. muscosa* responds to short-term salinity stress by quickly activating mechanisms which control volume. This is of adaptive significance to an organism inhabiting the intertidal zone, where salinity may change rapidly.

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Summary

1. The effects of external salinity changes on whole-animal volume, blood ions, and muscle-tissue water content of the chiton *Mopalia muscosa* were investigated. The data indicated that short-term low-salinity adaptation in the chiton *M. muscosa* is achieved by volume control mechanisms that are quickly activated.

2. Blood Na^+, Cl^-, K^+, and Mg^{2+} were isoionic to the SW concentration in salinities ranging between 60 and 125% SW. Blood Ca^{2+} concentration was hyperregulated in hyposmotic SW.

3. Regulation of cell volume occurred in salinity-stressed chitons, as water content of foot-muscle tissue was regulated in both hypo- and hyperosmotic media.

4. When exposed to hyposmotic SW the chitons at first (0–4 hr) gained weight; this was followed by a period in which the rate of weight gain approached zero (4–6 hr) and finally by a period of weight loss (6–24 hr) (volume regulation). Exposure to hyperosmotic SW resulted in weight loss and little volume regulation.

5. Volume control in hyposmotic media is accomplished, in part, by a loss of solute.

6. Low temperature, 7°C, decreased both the water influx and volume regulation.

7. Volume regulatory mechanisms are activated within 1 hr after exposure to 60% SW.

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